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PHYSICO-CHEMICAL ANALYSIS OF THE NATURAL FOOD COLOURS EXTRACTED BY USING NANO TECHNOLOGY

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ABSTRACT

The present study was conducted in the Department of Post Graduate And Research Center and Quality control lab, Rajendra Nagar, Hyderabad to synthesize the nano particles from selected fruits (papaya & black grapes) and vegetables (tomato & beet-root) and physical chemical characteristics of extracting nano food colors (NFCs) was assessed.

Selected fruits and vegetables are subjected to infrared drying (IR) and are grounded into a fine powder. The amount of powder obtained after drying the samples of papaya, black grapes, tomato and beet root was 8.0gm, 11.5gm, 6.0gm and 12.0gm, respectively for 100gm of fresh sample. Nano particles or NFCs were synthesized by the oxalate decomposition method. NFCs, thus obtained are subjected to scan under scanning electron microscope (SEM). The size of the NFCs ranged from 695.0-723.0 nm, 668.8-796.0 nm, 772.0-965.0 nm, and 859.0-991.0 nm in papaya, black grapes, tomato and beet root respectively. From the images scanned with SEM, it was observed that the NFCs are spherical in shape coated with zinc oxide. The NFCs thus obtained were assessed for physic chemical characteristics (Yield, Color quality, Concentration, Zinc and Bio active compounds).

KEYWORDS: Physic-Chemical Characteristics, Natural Food Colors, SEM, Infrared Drying and Nano Technology

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Research Article

INTRODUCTION

Color becomes the most sensitive part of any commodity not only for its appeal, but also it enhances consumer acceptability. The demand for natural colors is increasing day by day because of awareness of positive health benefit out of natural compounds (Chattopadhyay *et al*. 2008). The organic color stuffs are obtained from roots, stems, leaves, barks, flowers and berries of various plants and from certain insects and shellfish. The inorganic pigments are insoluble salts which are precipitated (Paul *et al*. 1996). There is a worldwide trend towards the use of natural additives and food colorant in food applications (Ghorpade *et al.* 1995). Nanotechnology describes materials, devices and systems with structures and components exhibiting new and significantly improved physical, chemical and biological properties, as well as the phenomena and processes, enabled by the ability to control properties of nano scale (Miyazaki and Islam, 2007). A range of nano techniques and materials are being developed to control over food character traits (Gardener, 2002). Inorganic nano materials are stable under harsh process conditions, but also generally regarded as safe to human beings and animals (Stoimenov *et al*. 2002; Fu *et al*. 2005).

Potential applications of nanotechnology are formulated of food products, food packaging applications and new materials for food equipments, new Sanitizers and also water purification (Enculescu *et al*. 2008).

MATERIALS AND METHODS

Raw Materials

The fruits (papaya & black grapes) and vegetables (tomato & beet root) were procured from local market. Chemicals used in this experimentation and analysis were of food grade, purchased from standard Indian companies.

Preparation

Fruits (papaya & black grapes) and vegetables (tomato & beet-root) of each 1kg were thoroughly washed in hot water and were cut into thin pieces. These pieces are placed in separate trays and were subjected to Infrared (IR) drying. After drying, the samples were cooled and grinded in a conventional grinder into fine powder.

Synthesis of Color Nano Particles

In the present study, NFCs were synthesized using dry powders from selected fruits (papaya & black grapes) and vegetables (tomato & beet root) by the oxalate decomposition method. Flow chart for synthesis of nano particles is given in Figure 1

Mix equimolar concentration of zinc source and oxalic acid

↓

Ground into powder and analyze to higher temperature like 600°C

↓

Powders of fruit and vegetables samples were sieved using 2 um sieve

↓

5% (v/v) extracts were prepared for the respective powders

↓

1% gum was prepared in 100ml conical flask

↓

0.2% nano scale zinc oxide solution was prepared

↓

1% gum solution was added to it while stirring

↓

Prepared 5% extract was added slowly with string and heating

↓

Final solution was made up to 100ml

↓

Solution was kept at room temperature for one hour for stabilization

Figure 1: Flow Chart for Synthesis of Nano Particles

Physic-Chemical Analysis

NFCs extracted were subjected to analysis, soon after their extraction for parameters like yield, color quality, concentration, zinc, total carotene, anthocyanin, lycopene and total beta lay contentedly. Yield of color was estimated using weighment method on dry weight basis. The color quality of the samples was estimated by using Hunterlab calorimeter (Color Quest XE hunter Lab, USA). The procedure for estimation of zinc was given by Garcia *et.al.* 1974. The concentration of the color was estimated by spectrophotometer as given by Jayaraman, 1996. Procedures for estimation of total carotene, anthocyanin, lycopene and total beta lain content were given by Ranganna (1986).

Plate 1: NFCs synthesized from Selected Fruits and Vegetables (Tomato, Beet Root, Papaya and Black Grapes)

Physical Quality Attributes

Determination of Particle Size

Scanning Electron Microscope (SEM) Protocol

Samples were exposed against 2.5% gluteraldehyde in 0.1M phosphate buffer (pH 7.2) for 1 hour at room temperature and dehydrated in silica desiccators for 1 hour. The processed samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of gold coat over the samples were done by using an automated sputter coater (Model-JEOL JFC-1600) for 3 minutes, and scanned under Scanning Electron Microscope (SEM - Model: JOEL-JSM 5600) at required magnifications (John and Lonnie, 1998).

FT-IR Measurements

The FT-IR measurements of the natural color encapsulated nanoparticles were carried out with TENSOR-27 (Bruker). To remove any free biomass residue or compound that is not the capping legend of the nano particles, the residual solution of 100 ml after the reaction was centrifuged at 15000 RPM for 15 min, and the resulting suspension was re-dispersed in 10 ml sterile distilled water. The centrifuging and re dispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain a dried powder. Finally, the dried nano particles were analyzed.

Statistical Analysis

The data were analyzed for difference of significance by ANOVA used CRD and CD values are presented

RESULTS AND DISCUSSIONS

Drying of Samples and Preparation of Fine Powder

The yield of the powder obtained after drying and grinding of samples is given in table 1

Table 1: Yield of Powder Obtained From the Given Samples after Drying and Grinding

Sample	Papaya	Black Grapes	Tomato	Beet root
Weight of powder in (gm)/1kg of fresh sample	80.0 (8.0)	115.0 (11.5)	60.0 (6.0)	120.0 (12.0)

***** Figures in parenthesis indicate percentages.

Particle Size and Structure

SEM

The NFCs synthesized from selected fruits (papaya & black grapes) and vegetables (tomato & beet root) were subjected to scan under Scanning Electron Microscope (SEM). The plates pertaining to the size and structure of the NFCs synthesized from selected fruits (papaya & black grapes) and vegetables (tomato & beet root) scanned with SEM are given in plate no's 4.1, 4.2, 4.3 & 4.4 for papaya, black grapes, tomato and beet-root, respectively. From the images scanned with a scanning electron microscope, it was found that the nano particles are spherical and agglomeralated in nature. They are found intact with the coating material (Zno). The coating material is highly thick holding the nano particles inside. It was also observed that the size of the color particles was in nanometers, where as the size of the coating material was in micrometers which showed that the zinc oxide was highly coated around the nano particles.

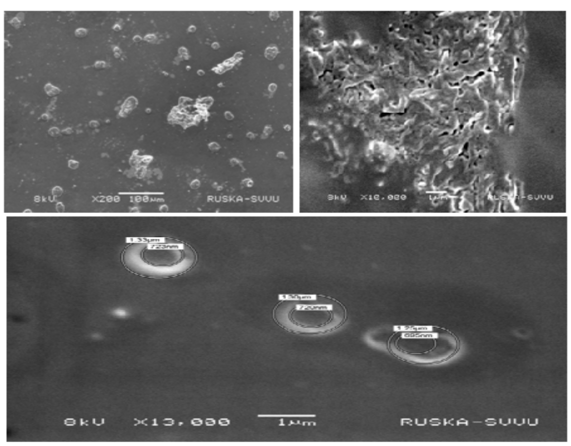


Plate 4.1: Size and structure of papaya's NFC

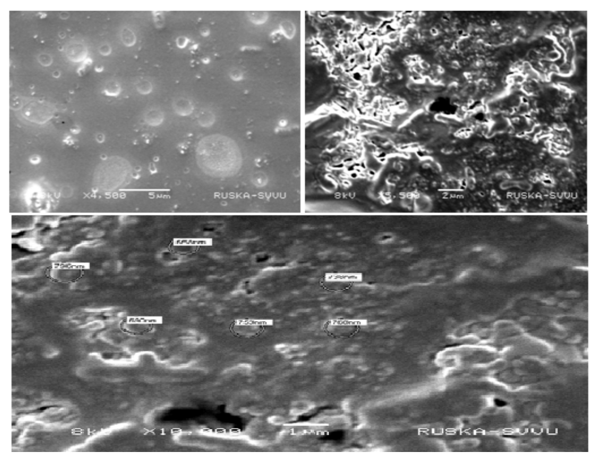


Plate 4.2 Size and structure of black grape's NFC

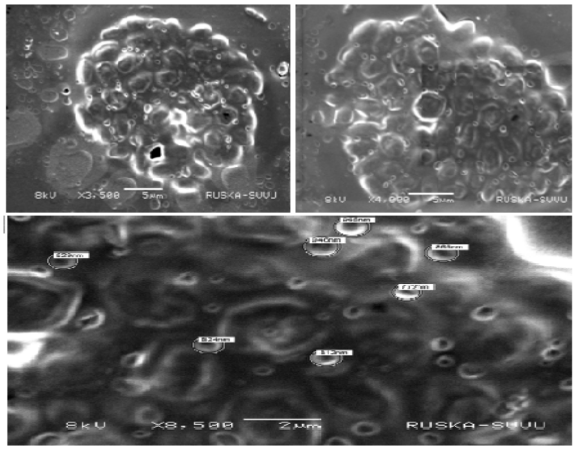


Plate 4.3: Size and Structure of Tomato's NFC

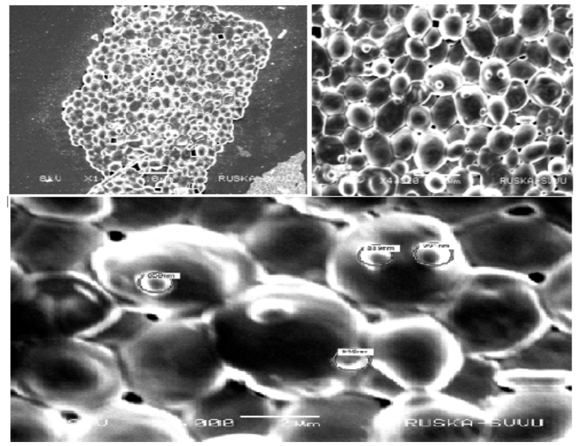


Plate 4.4 Size and Structure of Beet Root's NFC

The Size of the NFCs Obtained is given in Table 2.

Table 2: Size of NFCs Obtained from the Selected Fruits (Papaya & Black Grapes) & Vegetables (Tomato & Beet Root)

Sample	Range(nm)	Average (nm)
Papaya	695.0-723.0	713.0
Black grapes	668.0-796.0	736.0
Tomato	772.0-965.0	859.0
Beet root	859.0-991.0	925.0

The results in the above table indicated that the size of the NFCs varied from sample to sample. The size of the NFCs synthesized from papaya ranged from 695.0-723.0 nm, from black grapes (668.0-796.0 nm), from tomato (772.0-965.0 nm) and from beet root it ranged from 859.0-991.0 nm. The average size of the particles was 713.0, 736.0, 859.0 and 925.0 mm in papaya, black grapes, tomato and beet-root, respectively.

FT-IR Measurements

FT-IR results revealed that the absorption bands were present at 3349, 1635 cm^{-1} in NFCs of grape, tomato and papaya. The absorption band at 3349 cm^{-1} is assigned to the N–H group or primary amides which are present in the respective color extract. This also indicates the presence of alcohols with free OH. The absorption band 1635cm^{-1} corresponds to the presence of nitrites and tertiary amides. Whereas, the absorption band 2102 cm^{-1} , which is present in the sample Beet root NFC is assigned to the aromatic isonitriles, which is absent in the other samples tested. The results indicated that the color of the respective extract was clearly encapsulated by the zinc nano particles. According to Cross, 1960 for Tran's fatty acid absorption bands were present at 1000-950 cm^{-1} and for this fatty acid at 780- 680 cm^{-1}.

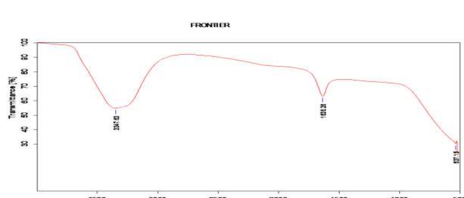


Figure 4.1: FT-IR Spectrum of Papaya's NFC

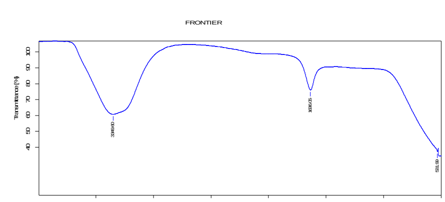
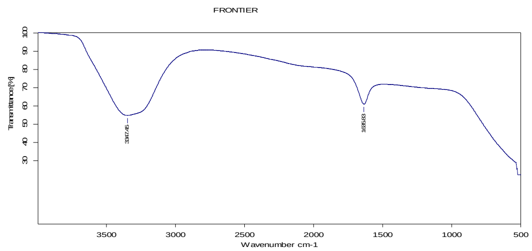


Figure 4.2: FT-IR Spectrum of Black Grapes's NFC



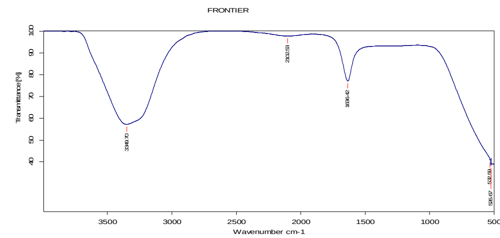


Figure 4.3: FT-IR spectrum of Tomato' NFC **Figure 4.4: FT-IR spectrum of Beet root's NFC**

DLS and Zeta Potential Measurements

The particle size (DLS) and zeta potential measurements of the prepared samples were carried out using Nano particles, SZ-100 (HORIBA). The hydrodynamic radius of the color encapsulated nano particles was recorded as 123nm, 232nm, 385nm; 1005nm corresponds to Papaya, Tomato, Grapes and Beet root, respectively. The sizes (hydrodynamic radius) of NFCs synthesized are in good correlation with the sizes measured under SEM. The higher zeta potential values (Grape: -40.5mV, Tomato: -36.5mV, Papaya: -32.7mV and Beetroot: -41mV) for all the samples tested indicate the high stability of the formed particles.

Yield

The yield of NFCs synthesized from selected fruits (papaya & black grapes) and vegetables (tomato & beet root) was calculated and recorded in the table 3.

Table 3: Yield of Nfcs Synthesized From Selected Fruits (Papaya & Black Grapes) and Vegetables (Tomato & Beet Root)

Sample	Weight of the Fresh Sample(Gm)	Weight of the Dry Powder(Gm)	Volume of Nano Particle Solution(Ml)
Papaya	100.0	8.0	160.0
Black grapes	100.0	11.5	230.0
Tomato	100.0	6.0	120.0
Beet root	100.0	12.0	240.0

The results illustrated in the table 3 indicated that the yield of the nano particle solution was 160.0 ml of 8.0 mg of dry powder of papaya, 230.0 ml of 11.5 mg of black grapes, 120.0 ml of 6.0 mg of tomato and 240.0 ml of 12 mg of beet-root for 100 mg of fresh sample.

Concentration

The results obtained for concentration of NFCs from selected fruits (papaya & black grapes) and vegetables (tomato & beet root) is given in the table 4.

Table 4: Concentration of Nfcs from Selected Fruits (Papaya & Black Grapes) and Vegetables (Tomato & Beet Root)

Sample	Papaya	Black grapes	Tomato	Beet root
Concentration	4.38	6.67	3.39	7.10

In beet root, the concentration of color was found to be higher (7.10), followed by black grapes (6.67), papaya (4.38) and least was for tomato (3.39). Between the vegetable samples the concentration of color was found to be higher in the Beetroot (7.10) and lower in tomato (3.39). In fruit samples the concentration of color was found to be higher in black

grapes (6.67) followed by papaya (4.38). The values obtained in the present study for concentration are much higher compared to the value reported by Priya mandhana *et* al, (2007). This may be mainly due to the synthesis of the color nano particles with very small size and large surface area to volume ratio.

Color Quality

The color quality of NFCs synthesized from selected fruits (papaya & black grapes) and vegetables (tomato & beet root) is given in table 5.

Table 5: Color Quality of Nfcs Synthesized From Selected Fruits (Papaya & Black Grapes) and Vegetables (Tomato & Beet Root)

Sample	Light (L)	Hue (a)	Brightness (b)
Papaya	14.55	3.33	4.59
Black grapes	16.56	3.59	8.53
Tomato	14.26	2.73	5.45
Beet root	13.36	4.56	5.34

The color value was mainly determined in the form of light, hue and brightness. Among the vegetable samples, lightness was found to be higher for NFC with tomato (14.26%) followed by NFC with beet root (13.36%). In the fruit samples lightness was found to be higher in NFC with black grapes (16.56%) and lower in the NFC with papaya (14.55%). The values for lightness for all the NFCs were extended nearly towards '0' value, i.e., black indicating good color quality. The + a (Hue) value which indicates more of redness and less of greenness was higher for Beetroot NFC (4.56%) compared to the NFC with tomato (2.73%) among vegetable samples. In fruit samples hue, value was higher for NFC with black grapes (3.59%) compared to the NFC with papaya (3.33%). Between the vegetable samples the brightness was found to be higher for NFC with tomato (5.45%) and lower for NFC with beet root (5.34%) and in fruit samples higher for NFC with black grapes (8.53%) compared to the NFC with papaya (4.59%).

Zinc

The nano particles synthesized from selected fruits (papaya & black grapes) and vegetables (tomato & beet root) in the present study are coated with zinc oxide. Hence, zinc was estimated in the NFCs to know the amount of zinc that is present in the given nano particle solution. As zinc rich foods tend to be expensive, the daily intakes of zinc were much lesser to the requirement. The recommended daily intake of zinc is 15mg/day. The amount of zinc in the given NFCs of selected fruits (papaya & black grapes) and vegetables (tomato & beet root) is given in the table 6.

Table 6: Zinc Content in the Nfcs Synthesized From Selected Fruits (Papaya & Black Grapes)And Vegetables (Tomato & Beet Root)

Sample	Papaya	Black Grapes	Tomato	Beet Root
Zinc content(mg/100gm)	3.94	6.81	2.95	7.51

The results illustrated in the table 6 indicated that the zinc content in NFCs synthesized from papaya was 3.94 mg/100gm, 6.81mg in black grapes, 2.95mg in tomato and 7.51 mg/100gm in beet root. In fresh samples of tomato and beet root, the zinc content was 0.41 and 0.91, respectively, whereas in papaya and black grapes, it was not reported (Gopalan *et al.* 2007). When compared to the above values, it was observed that, the Zinc content of NFCs synthesized was higher, indicating the significance of NFCs in enhancing the Zinc content of the food products. Zinc and zinc oxide were used as nutritional additives and antimicrobial agents in food packaging (Brunner, 2006). Hence, these NFCs also can be used as nutritional additives and also as antimicrobial agents.

Bioactive Compounds

The quantity of bio active compounds was estimated in the NFCs synthesized from selected fruits such as total carotenes (papaya) & anthocyanins (black grapes) and vegetables such as lycopene (tomato) & battalions (beet root) and results are given in table 7.

Table 7: Quantity of Bio Active Compounds in Nfcs Synthesized from Selected Fruits (Papaya & Black Grapes) and Vegetables (Tomato & Beet Root)

Bioactive Compounds	O.D Value	Content In (100gm)
Total carotenes (papaya)	0.31	92.16 µg
Anthocyanins (black grapes)	0.27	118.91 mg
Lycopene (tomato)	0.125	4.68 mg
Betalains (beet root)	2.2	5.28 mg

Results in the table 7 indicates that the total carotene content in the NFC with papaya was 92.16 µg/100gms. The anthocyanin content in NFC with black grapes was 118.91 mg/100gm. The lycopene content in the NFC with tomato was 4.68 mg and betalain content in the NFC with beet root was 5.28 mg/100gm.

CONCLUSIONS

Thus, it can be concluded that it is feasible to synthesize NFCs from dehydrated powders of selected fruits (papaya and black grapes) and vegetables (tomato and beet root) with good physic-chemical (yield, color quality, concentration, zinc and bio active compounds) properties.

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